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Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria

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Abstract

Sixty plant species were collected in the Ivory Coast on the basis of an ethnobotanical literature using the following three criteria: activity against worms, diarrhoea and/or abdominal pain. Eighty six plant extracts were prepared using 90% ethanol and tested for potential anthelminthic activities with a larvicidal test of *Haemonchus contortus*. 25.6% of the extracts showed a high activity, 12.8% were active or marginally active and the remaining 61.6% were inactive. The fact that a relatively high percentage of the plants species (50%) had an activity can be explained by the initial preselection of the plants on the basis of ethnobotanical indications.

Keywords: Anthelmintic; Sheep; *Haemonchus contortus*; Medicinal plants; Ethnobotany

1. Introduction

Infections of animals with gastrointestinal nematodes are a world wide problem. These parasites cause frequently important economic losses due to mortality in the case of heavy infection. In addition, chronic infections cause a lowering of productivity, fertility, growth, milk and meat production (Holmes, 1985; Fox, 1997). The anthelminthics discovered and used since the '60s have permitted to limit the problem until the appearance of resistance and even multi-resistance in nematode populations (Sangster, 1999; Zajac and Gipson, 2000). Actually, the situation becomes very problematic or even critical in certain regions of the globe (Waller, 1997; Van Wyk et al., 1999; Jackson and Coop, 2000).

Several control measures are available (Waller, 1999) like vaccination (Bain, 1999; Newton and Munn, 1999; Smith, 1999), breeding of resistant sheep races (Gray, 1997), improvement of food quality (Wallace et al., 1998; Datta et al., 1999; Coop and Kyriazakis, 1999; Knox, 2002) and better methods of breeding and dairy farming (Stear et al., 1998; Barger, 1999; Niezen et al., 1999). In addition, biological measures are being developed (de Gives et al., 1999; Larsen, 1999; Pena et al., 2002) and the use of anthelminthics already present on the market has to be optimised (Hennessy, 1997; Smith et al., 1999; Van Wyk and Bath, 2002). In spite of these efforts, the search for new nematocidal substances remains a priority (Geary et al., 1999; Witty, 1999).

Since a long time, mankind has developed throughout the world a traditional medicine based on the knowledge of medicinal plants. This knowledge got enriched over numerous generations due to experimentation but also through observation of animal behaviour. Most of the time, this information is only orally inherited and is therefore in danger of being lost in favor of modern medicine. However, it represents for the local population a possibility of simple and cheap treatment. In addition, it is a source of potentially important new pharmaceutical substances. The interest and urgency of ethnobotanical research is thus obvious (Schillhorn van Veen, 1997; Hammond et al., 1997). Several molecules with antiparasitic activities, as for example quinine and artemisinin, have been identified in plants and are actually widely used

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(Kayser et al., 2003), others show promising nematicidal activities (Al-Qarawi et al., 2001; Onyeyili et al., 2001).

Only a few ethnobotanical studies evaluate the traditional veterinary medicine (Lans and Brown, 1998a,b; Vieira et al., 1999). Only a few publications treat the use of medicinal plants in the veterinary setting of the African continent (Aké Assi, 1992; Bizimana, 1994; Fakae et al., 2000; Nfi et al., 2001), but studies verifying the presumed activities are rare.

The resistance against medically active substances is particularly pronounced in the case of gastrointestinal nematodes of sheep and goats (Sangster and Gill, 1999) and especially in the case of *Haemonchus contortus* Rudolphi (1803), which is an hematophagous nematode of veterinary importance throughout the world. In West Africa this gastrointestinal parasite is dominant in ruminants (Achi et al., 2003).

Haemonchus contortus develops as free living nematodes from the eggs to the infective L3 stage. The ruminants become infected with L3 while grazing and the further development of the parasite passes via several moults to the adult stage, which is finally localised in the abomasum. The female of *Haemonchus contortus* produces 5000–10,000 eggs per day released with the host faeces. The free living stages of this parasite can therefore be used as a model for anthelminthic bioassays to test plant extract activities.

In order to identify anthelminthic activities in plants different strategies can be proposed: random screening of a large number of extracts, focusing on one species, genus or family, testing all parts of a specific plant for potential activities or finally screening a selected number of plants on the basis of ethnobotanic information (Fabricant and Farnsworth, 2001; Rates, 2001). The last approach was selected for this study, due to its advantage of rapidly identifying a limited number of interesting plants. However, a certain risk to miss some plant species with potential anthelminthic activities has to be admitted, because some plants might not have been documented in the traditional medicine.

2. Material and methods

2.1. Plants

Several ethnobotanical books on traditional, human or veterinary medicine in Africa have been consulted (de Koning, J., Rapport de stage, 1970, ORSTOM; Abbiw, 1990; Aké Assi and Guinko, 1991; Aké Assi, 1992; Iwu, 1993; Sofowora, 1996; Weiss, 1997), the major source being the book on “Traditional veterinary practice in Africa” (Bizimana, 1994). The choice of the plants and their parts was based on three criteria, namely active (a) against worm affections in general (worms in general, round worms, Guinea worms, flatworms), (b) against diarrhoea and dysentery and (c) against abdominal pain. Plant parts were finally collected, which were cited for all possible combinations of these three criteria.

Sixty plant species were collected in the regions of the savannah and the secondary rain forest of the Ivory Coast between October and December of the year 1996. Voucher specimens were deposited at the “Centre Suisse de Recherches Scientifiques”, Adioupoudoumé, Ivory Coast, in the National Floristic Center of the Ivory Coast and in the Botanical Institute of the University of Neuchâtel, Switzerland. Samples of *Omphalocarpum elatum* Miers, *Ceiba pentandra* (L.) Gaertn. and *Garcinia kola* Heckel were tested, but could not be added as voucher specimens to the herbarium.

2.2. Preparation of extracts

A total of 86 samples from leaves, bark and/or roots of the 60 plant species were used: 25 g of dry powder of each sample was extracted during 12 h in 250 ml of 90% ethanol under constant agitation and at room temperature. After filtration the ethanol was evaporated under vacuum in a Rotavapor. The extract was lyophilized and stored at −20 °C.

2.3. Production of Haemonchus contortus eggs

Six to nine months old female sheep of the local Sahelien and Djallonké race were treated by a single dose of 2 ml of Levamisol, 3.75% (LAPROVET) per 10 kg weight and kept pairwise during 1 month in a box with a wooden bar bottom. The absence of any gastrointestinal parasites in feces was microscopically verified. Every animal was then infected orally with a single dose of 1500–3000 third stage larvae of *Haemonchus contortus*. Eggs were collected three weeks after the infection and the eggs per gram (epg) was daily determined. The feces, collected during a 12 h period, were diluted in water, filtered through gauze and then through a sieve with a mesh size of 32 μm. The sediments were resuspended in a saturated solution of glucose and centrifuged at 1500 rpm during 10 min. The supernatant was filtrated through sieves with a mesh size of 50 and 32 μm. The egg suspension was used on the same day.

2.4. In vitro anthelmintic test

Thirty mg of the dry plant extracts were solubilized in 1 ml dimethylsulfoxide and tested 2–3 times in duplicates in the bioassay. 20 μl of serial aqueous dilutions ranging from 1.7 to 1.3×10^{-3} mg/ml were used in duplicate on 96 multiwell plates. 140 μl of 2% agar with 1% Fungizone kept at 50 °C were used to overlay the extracts. After solidification, about 70 eggs of *Haemonchus contortus* were added to each well. The plates were incubated for 24 h at 25 °C with a 100% relative humidity.

Twenty μl of a culture medium (composition: proteose peptone and yeast extract, 0.5 g each in 100 ml of Earls salt, 10× diluted in physiological salt solution) was then added in order to favour bacterial growth used as the major source of nutrients by the free living larval stages.

The successful hatching of the larvae was microscopically verified in the control wells and the degree of hatching was determined in the wells containing the plant extracts.

Six days after inoculation the number of nematodes present, the degree of their development (proportion of nematodes in the larval stages L1, L2 resp. L3) and the viability of the larvae were microscopically evaluated in each well. A high nematicidal activity was defined when a 95–100% total larval mortality was observed. Eighty to ninetyfive percent mortality was considered as an intermediate activity and a low activity was monitored with a mortality of 60–80%.

Twenty μl Moxidectine and Fenbendazole, two standard anthelminthics, were used as positive controls (from a stock of 7.5 mg/ml DMSO in aqueous dilutions of 0.28 to 1.7×10^{-7} mg/ml). As negative controls wells were filled with 20 μl deionized water.

3. Results

From the totally 60 plant species collected in the field (Table 1), seven have been cited for their use against worms, diarrhoea and abdominal pain together (Table 4). Eighteen species were recommended against worms and diarrhoea, five species against worms and abdominal pain together and five against diarrhoea and abdominal pain. Twelve species were indicated against worms only, the rest against one of the other two criteria.

In the control wells of the bioassay the hatching rate of *Haemonchus contortus* was generally between 90 and 100%, and only in one assay the rate was between 85 to 90%. The mean development rate to the L3 larval stage was between 65 to 75%. A 100% mortality was found at the L2 stage in the wells containing the anthelminthics Fenbenzadole or Moxidectine up to a dilution of 1.67×10^{-7} mg/ml.

Among the 86 samples 33 showed anthelminthic activities (Table 2). Twenty two plant extracts caused a mortality of 95–100% of the larvae at the last active dilution corresponding to 0.43–1.7 mg/ml of the extracts. A category of nine extracts, with a concentration range of 0.0963–1.7 mg/ml, induced a mortality of 80–94% and finally two extracts caused a 60–79% mortality in the range of 0.848–1.7 mg/ml. Higher concentrations of the extracts did not result in increased mortality rates.

4. Discussion

Three criteria (active against worm affections in general, diarrhea/dysentery and abdominal pain) frequently used in the ethnobotanical literature (for example Bizimana, 1994) were used to select plants with a potential active substance against nematodes. Priority was given to plants indicated against worms, because diarrhoea and abdominal pain on their own can be due to either an infection by gastrointestinal worms or numerous other troubles of the digestive system. An exhaustive list of plants was prepared (unpublished data) on the basis of the traditional, human and veterinary medicine literature of Africa (de Koning, J., Rapport de stage, 1970, ORSTOM; Abbiw, 1990; Aké Assi and Guinko, 1991; Aké Assi, 1992; Iwu, 1993; Bizimana, 1994; Sofowora, 1996 and Weiss, 1997). The final choice of the species collected was determined by the availability of the plants in the field.

Limiting the screening on the information present in traditional medicine has the inconvenience that unknown activities will not be detected. In addition, the degree of an activity can vary in various parts of a plant (for instance buds, or young, aging or desiccated leaves) and during different time points (morning, noon or evening, beginning or end of the dry season, etc). The medications proposed by traditional medicine are frequently a mixture of several plants prepared in different manners (fresh parts, exudates, ashes, teas, decoction etc.). There are only few scientific indications available which demonstrate a priori the efficacy of such traditional medications. Ethnobotanical information, as precious as it may be, must be verified using a standardized test system.

The use of *Haemonchus contortus* as a test system is justified by the fact that this nematode is an intestinal parasite of economic importance in the farming of small ruminants. Its free larval development, also under in vitro conditions, allows a simple standardization based on mobility and morphological parameters.

The use of Fenbendazole and Moxidectine, two commonly used anthelminthic molecules as positive controls, allowed the validation of the test system in the absence of a standardized plant extract. It is obvious that the plant samples are tested at higher concentration levels, since the components are not purified and the active molecules represent probably only a fraction of the total sample, explaining the factor of 10^{-7} of difference between the standards and the plant extracts (Rates, 2001).

The results of the anthelminthic activities obtained within a family or a species deviated sometimes from the expected outcome: for instance, *Rauvolfia vomitoria* Afzel., a species very often cited and thus promising, was finally found to be inactive. On the other hand *Parinari excelsa* Sabine, cited only once to be indicated against diarrhoea and thus a priori of little interest, showed a high activity in the bioassay. Certain plants showed an activity in all parts tested, as in the case of the leaves and the bark of *Terminalia avicennioides* Guill. & Perr. *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth., however, had strong activities in the leaves, moderately in the bark and no activity in the roots. Such results are not surprising in view of the ethnobotanical information based exclusively on empirical therapeutic indications.

In fact, among the highly active plant species identified with our test system, *Ximenia americana* L., *Khaya senegalensis* (Desv.) A. Juss. and *Annona senegalensis* Pers. were indeed most frequently cited for the use against worms and diarrhea (Table 2). 25.6% of the plant extracts and 31.7% of species tested showed relatively high activities. If the extracts or species with moderate to low activities (leading to 60–95% mortality) are included, the percentages of active samples increase to 30.4 resp. 50% (Table 3). The 30 plant species with an anthelminthic activity were analysed

Table 1
List of the plant species selected using ethnobotanical informations

Family/species/(voucher specimen[a])	Plant part cited[b]	Traditional use[c]		
		Worms	Diarrhea	Abdominal pains
Aloaceae				
Aloe buettneri Berger (387309)	Leaf	1, 4		
Anacardiaceae				
Pseudospondias microcarpa (A. Rich.) Engl. (387186-8)	Leaf, root	4		
Annonaceae				
Annona senegalensis Pers. (387281-3)	Bark, fruit, leaf, root, seed, stem root, stem	1, 2, 4	1, 3, 4	
Xylopia aethiopica A. Rich. (387234/93)	Bark, fruit, seed	1, 4, 5	1, 5	
Apocynaceae				
Rauvolfia vomitoria Afzel. (387300-1)	Bark, leaf, root, stem root	8	1, 6	1, 8
Asteraceae				
Ageratum conyzoides Linn. (387312)	Leaf, root, whole plant			5, 8
Erigeron floribundus (H.B. & K.) Sch. Bip. (387296-7)	Leaf			8
Bidens pilosa L. (387311)	Leaf		4, 6	
Vernonia nigritiana Oliv. & Hiern. (387202-4)	Root		1	
Bombacaceae				
Adansonia digitata L. (387322-3)	Leaf, seed, fruit pulp	1, 2	1, 3, 4	
Ceiba pentandra (L.) Gaertn. (−)	Bark, leaf, root	4	1	
Caesalpiniaceae				
Cassia occidentalis L. (387287-8)	Leaf, root, seed	1, 2, 4		
Cassia sieberiana var villosa A. Chev. (387213-4)	Leaf	1		1
Cassia tora L. (387290-2)	Root	4		
Piliostigma thonningii (Schumach.) Milne-Redh (387279-80)	Bark, bud, leaf		1, 3	3
Caricaceae				
Carica papaya L. (387212/7310)	Bark, fruit, leaf, root, sap, seed, shoot, stem root	3, 4, 5		3, 8
Chrysobalanaceae				
Parinari curatellifolia Planch. ex Benth. (387209-11)	Stem	4		
Parinari excelsa Sabine (387260-2)	Fruit		1	
Clusiaceae				
Garcinia kola Heckel (−)	Fruit, seed	1	5	
Combretaceae				
Anogeissus leiocarpa (DC.) Guill. & Perr. (387239-41)	Bark, coal, fruit, leaf, root, seed, stem	2, 4, 5	1, 3	
Terminalia avicennioides Guill. & Perr. (387286)	Leaf, root, stem root	2, 4	1	
Connaraceae				
Cnestis ferruginea Vahl. ex DC (387202-07)	Leaf, root, stem root		1	8
Ebenaceae				
Diospyros mespiliformis Hochst. ex A. DC. (387275-8)	Ba, Lf, Sb	2, 4		
Euphorbiaceae				
Flueggea virosa (Roxb. ex Willd.) Voigt (387273-4)	Leaf, stem	1		
*Jatropha curcas*L. (387249)	Leaf ashes, bark, leaf, root, seed	1, 3, 5	1, 5	
Ricinus communis L. (387263-5)	Leaf, seed	1	4	
Fabaceae				
Dalbergiella welwitschii (Bak.) Bak. F. (387319-21)	Leaf	1	8	
Erythrina senegalensis A. DC. (387189)	Bark, root	4	1	
Humiriaceae				
Sacoglottis gabonensis (Baill.) Urban (387302-3)	Bark			8
Hypericaceae				
Harungana madagascariensis Lam. ex Poir. (387215-6)	Bark, berry, bud, leaf, root, stem	1, 4	1, 6	1
Lecythidaceae				
Napoleonea leonensis Hutch. & Dalz. (387228-9)	Leaf		8	
Malvaceae				
Sida acuta subsp carpinifolia (L. f.) Borss. (387270-1)	Leaf	4		

Table 1 (*Continued*)

Family/species/(voucher specimen[a])	Plant part cited[b]	Traditional use[c]		
		Worms	Diarrhea	Abdominal pains
Melastomataceae				
Heterotis rotundifolia (Smith) Jac.-Fél. (387176-7)	Leaf	5		6
Meliaceae				
Khaya senegalensis (Desv.) A. Juss. (387217-9)	Bark, leaf, stem	1, 2, 4, 5	4, 5	1
Mimosaceae				
Albizia anthelmintica cf Brongn. (387298-9)	Bark	4	4	
Pentaclethra macrophylla Benth. (387190)	Bark		1	8
Moraceae				
Ficus exasperata Vahl (387245)	Leaf	1		1
Musanga cecropioides R. Br. (387178-9)	Coal, stem bark, wood	8	8	
Myristicaceae				
Pycnanthus angolensis (Welw.) Warb. (387225-7)	Root	1, 8		
Olacaceae				
Ximenia americana L. (387201)	Fruit, leaf, root, stem root	1, 5, 7	1, 3, 4, 7	3
Opiliaceae				
Opilia amentalea Roxb. (387180/387237-8)	Leaf, root	1, 4	4	
Pandaceae				
Microdesmis puberula Hook. F. ex Planchon (387250-2)	Bark, leaf	1	1, 8	
Passifloraceae				
Adenia lobata (Jacq.) Engl. (387313-4)	Leaf	8		
Piperaceae				
Pothomorphe umbellata L. (387235-6)	Fruit, root, stem		1, 5	8
Poaceae				
Pennisetum purpureum Schumach. (387289)	Leaf, stem	4		
Polygalaceae				
Securidaca longipedunculata Fres. (387258-9)	Leaf, root	4		
Rhamnaceae				
Ziziphus mauritiana Lam. (387181-5)	Bark, leaf, root	1	1	
Rubiaceae				
Crossopteryx febrifuga (Afzel. ex G. Don) Benth. (387197-00)	Bark, leaf, root, stem bark	1, 3	1, 3	1
Mitragina ciliata Aubrév. & Pellegr. (387284-5)	Bark, leaf	8	1, 5	
Mitragina inermis (Willd.) O. Ktze. (387191-5)	Bark, leaf		2, 4	1
Morinda longiflora G. Don (387230-3)	Fruit, leaf, root	5	5	8
Nauclea latifolia Sm. (387307-8)	Bark, leaf, root	4, 6	1, 6	1
Rutaceae				
Zanthoxylum xanthoxyloides (Lam.) Zepernick & Timber (387196/387208)	Fruit, leaf, pulp, stem bark	1, 5	1	1
Sapotaceae				
Omphalocarpum elatum Miers (−)	Bark			8
Scrophulariaceae				
Scoparia dulcis L. (387318)	Leaf			8
Solanaceae				
Nicotiana tabacum L. (387266-9)		4		
Sterculiaceae				
Cola caricaefolia (G. Don) K. Schum. (387246-8)	Leaf		8	8
Ulmaceae				
Trema orientalis (L.) Blume (387242-3)	Leaf, root	1, 3, 6	1	
Zingiberaceae				
Aframomum danielli (Hook. f.) K. Schum. (387294-5)	Leaf			8
Aframomum sceptrum[c] (Oliv. & Hanb.) K. Schum. (387315-6)				

[a] Identification number, herbarium at the University of Neuchâtel, Suisse. Three plant species were tested, but not deposited as voucher specimens (−).
[b] Literature sources used for the plant selection: (1) Abbiw; (2) Aké-Assi; (3) Aké-Assi & Guinko; (4) Bizimana; (5) Iwu; (6) de Koning, J. Rapport de stage. 1970. ORSTOM; (7) Sofowora; (8) Weiss.
[c] *Aframomum sceptrum* (Oliv. & Hanb.) K. Schum. was collected without a particular ethnobotanical indication in order to compare the activity in within the same genus.

Table 2
Activities of plant samples tested

Plant species	Number of citations found for each criteria[a]			Total citations	Plant parts tested	Minimal active concentration (mg/ml)
	W	D	A			
Highly active with 95–100% larval mortality						
Annona senegalensis Pers.	3	3	–	6	Stem bark	0.43
					Bark	0.43
Anogeissus leiocarpus (DC.) Guill. & Perr.	3	2	–	5	Stem bark	0.43
Crossopteryx febrifuga (Afzel ex G. Don) Benth.	2	2	1	5	Leaves	0.43–0.849
Flueggea virosa (Roxb. ex Willd.) Voigt	–	1	–	1	Leaves	1.7
Harungana madagascariensis Lam. ex Poir.	2	2	1	5	Bark	0.849
Jatropha curcas L.	3	2	–	5	Root	0.849
Khaya senegalensis (Desv.) A. Juss.	4	2	1	7	Bark	0.849–1.7
Musanga cecropioides R. Br.	1	1	–	2	Stem bark	0.849
Napoleonea leonensis Hutch. & Dalz.	–	1	–	1	Leaves	1.7
Omphalocarpum elatum Miers	–	–	1	1	Bark	0.849
Parinari excelsa Sabine	–	1	–	1	Bark	0.849
Piliostigma thonningii (Schumach.) Milne-Redh	–	2	1	3	Bark	0.849
Sacoglottis gabonensis (Baill.) Urban	–	–	1	1	Bark	0.43–0.849
Securidaca longipedunculata Fres.	1	–	–	1	Leaves	0.849
					Root	0.849
Terminalia avicennioides Guill. & Perr.	2	1	–	3	Bark	0.849
					Leaves	0.849
Trema orientalis (L.) Blume	3	1	–	4	Root	0.849
Ximenia americana L.	3	4	1	8	Root	0.849
Xylopia aethiopica (Dun.) A. Rich.	3	2	–	5	Bark	0.43
Ziziphus mauritiana Lam.	1	1	–	2	Leaves	1.7
Active with 80–95% larval mortality						
Adansonia digitata L.	2	3	–	5	Leaves	0.43–0.849
Cassia sieberiana DC.	1	–	–	1	Leaves	0.849
Ceiba pentandra (L.) Gaertn.	1	1	–	2	Bark	0.0963/1.7
Crossopteryx febrifuga (Afzel. Ex G. Don) Benth.	2	2	1	5	Bark	0.0963
Diospyros mespiliformis Hochst ex A. DC.	2	–	–	2	Bark	0.849
Mitragina inermis (Willd.) O. Ktze.	–	2	1	3	Bark	0.849–1.7
Parinari curatellifolia Planch. ex Benth.	1	–	–	1	Stem	0.849
Pycnanthus angolensis (Welw.) Warb.	2	–	–	2	Bark	1.7
Zanthoxylum xanthoxyloides (Lam.) Zepernic	2	1	1	4	Leaves	0.849–1.7
Marginally active with 60–80% larval mortality						
Microdesmis puberula Hook. ex Planch.	1	2	–	3	Leaves	1.7
Morinda longiflora G. Don	1	1	1	3	Root	0.849–1.7

[a] W: indicated against worm infections in general; D: indicated for diarrhoea; A: indicated against abdominal pain.

according to the three ethnobotanical criteria used (Table 1) and some of these plant species were cited several times. Twenty one of them were recommended to be used against worm affections in general (Table 4) corresponding to 50% of all plants cited ($N = 42$). The most efficient indications in the category of worms in general were the citations of a given plant species to be used against worm affection only, the combination against worms and diarrhoea, and those against worms, diarrhoea and abdominal pain together. In order to select a plant species the combination of the three main criteria as well as the combination of the criteria against worms and diarrhoea renders the best results (Table 4). This seems logical because diarrhoea/dysentery and abdominal pain are symptoms frequently associated with the presence of worms.

However, if the citation of a plant against diarrhoea/dysentery can be a useful indication similar to the one against worms, the abdominal pain as a parameter on its own is less important. A statistical analysis is not useful, because of the intentionally biased search of plants. In order to make a statistical evaluation a parallel random large scale sampling procedure would be necessary.

Table 3
Plant extracts and plant species with larvicidal activities

Categories	Number	Percentage
Extracts		
Total number of extracts tested	86	100
Highly active extracts	22	25.6
Active and marginally active extracts	11	12.8
Inactive extracts	53	61.6
Species		
Total number of species tested	60	100
Highly active species	19	31.7
Active and marginally active species	11	18.3
Inactive species	30	50

Table 4
Activities of plant species in relation to the three main selection criteria

Criteria	Total plant species	Highly active	Percentage	All active categories	Percentage
Worm affections in general	42	14	33.3	21	50
Worms only	12	2	16.7	5	41.7
Worms and diarrhoea	18	8	44.4	10	55.5
Worms and abdominal pain	5	0	0	2	20
All three criteria together	7	4	57.1	4	57.1
Diarrhoea in general	36	15	41.7	18	50
Diarrhoea only	6	2	33.3	2	33.3
Diarrhoea and abdominal pain	5	1	20	2	40
Abdominal pain in general	23	7	30.4	10	43.5
Abdominal pain only	6	2	33.3	2	33.3

A plant extract showing no anthelminthic activity can not be considered as being completely inactive. Activities resulting of mixtures of different plant species or from a special preparation procedure of the plant can be absent in isolated extracts. The extraction method using 90% ethanol does not guarantee a complete extraction of potential active molecules. In addition, the larval development test has also its limits, because it does not allow to detect all potential biological activities against helminths (Kotze et al., 1999; Varady and Corba, 1999). It would also be indispensable to test again extracts studied in this work using samples obtained from other individuals of the same species collected at different seasonal periods of the year and from other regions in order to verify the results before proceeding to more detailed studies.

Our results should therefore permit the verification of the initial hypothesis that screening based on ethnobotanical indications will allow an efficient discovery of active species compared with a random screening (Fabricant and Farnsworth, 2001).

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